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(E) PROTEIN LOCALIZATION and tissue distribution

The human histidine protein phosphatase gene is localized at chromosome 9 (9q33 -Tel, marker sts-N90764, interval D9S159-qTEL).

Sources for the expression of cDNAs were used:

- 5 Brain, breast, CNS, colon, foreskin, germ cell, heart, kidney, liver, lung, muscle, pancreas, parathyroid, pooled, prostate, spleen, testis, thyroid, tonsil, uterus, whole embryo.

Analyzing the distribution of histidine protein phosphatase mRNA showed an increased level in normal tissues as heart, kidney, liver, pancreas, skeletal muscle and testis (~~Figure 9 a, b~~).

(Figures 8a-8c)

(F) Anti-Histidine phosphatase antibodies

- 15 Anti-Histidine phosphatase antibodies were generated against three distinct regions of the protein, namely the n-terminal, the middle and the c-terminal part of the molecule. For this purpose three peptide sequences were chosen:

peptide 1 - QIPDVIDSD GVFKYV (16aa, SEQ. No. 9);

peptide 2 - CLGGGRISHQ SQDK (14aa, SEQ. No. 10);

peptide 3 - CTEKIKAKYP DYEY (14aa, SEQ. No. 11).

- 20 The peptides were synthesized using standard FMOC-chemistry. For immunization the peptides were injected (4 injections) each into two rabbits and four blood samples were taken. Final bleeding was taken after ca. 3 month. The generated antibodies are usefull for detection and localization of the histidine phosphatase.

- 25 Furthermore, the different regions within the molecule can be analyzed individually. Especially the highly conserved central part of the histidine phosphatase containing the following amino acid sequence:

DCECLGGGRISHQSQD (SEQ. No. 3)

is assumed to contain the active site responsible for the proteins function in vivo.

- 30 The anti-peptide antibody against this region is for inhibitory or neutralizing use.

The characteristics of the histidine protein phosphatase can be summarized as follows:

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